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(54) Title: FRAMESHIFT MUTANTS OF BETA–AMYLOID PRECURSOR PROTEIN AND UBIQUITIN–B AND THEIR USE

(57) Abstract

Frameshift Mutants β–Amyloid precursor peptides and mutant ubiquitin–B associated with Alzheimer's disease and Down syndrome eliciting T cellular immunity for use in compositions for the treatment and/or prophylaxis of Alzheimer's disease and/or Down syndrome.

FRAMESHIFT MUTANTS OF BETA-AMYLOID PRECURSOR PROTEIN AND UBIQUITIN-B AND THEIR USE

11/4/03

The present invention relates to peptides for treatment and/or prophylaxis of Alzheimer's disease and Down syndrome.

Alzheimer's disease and treatment of Down syndrome are both associated with frameshift mutations occurring at the transcriptional level or by posttranscriptional editing of RNA during the encoding of β-Amyloid precursor protein (βAPP) and ubiquitin-B (Ubi-B). Such frameshift mutations give rise to mutant βAPP and Ubi-B protein products which are characterised by aberrant protein sequences at the carboxyl terminus. Peptides covering, either completely or parts of, the aberrant parts of mutant βAPP or Ubi-B protein products elicit T cellular immunity and can therefore be useful in compositions for the treatment of Alzheimer's disease and Down syndrome. Further the peptides of this invention can be used as a prophylaxtic anti-Alzheimer's disease vaccine.

The invention also relates to DNA sequences encoding peptides corresponding to aberrant βAPP and Ubi-B protein sequences found in Alzheimer's disease and Down syndrome patients, and to vectors comprising at least one insertion site containing a DNA sequence encoding at least one such peptide.

Further the invention relates to methods for the treatment and/or prophylaxis of Alzheimer's disease by administration of at least one mutant βAPP and/or Ubi-B peptide or a recombinant virus vector comprising at least one insertion

In Alzheimer's and Down syndrome patients, intracellular and extracellular deposites of proteins in tangles, neurophil threads and neuritic plaques are correlated with neuronal dysfunction leading to dementia (R.D.Terry et al in *Alzheimer Disease*, R.D.Terry, R.Katzman, K.L.Bick, Eds. (Raven, New York, 1994) pp. 179-196). These protein deposits have been shown to contain forms of β amyloid precursor protein (βAPP) and ubiquitin-B (Ubi-B) that are aberrant in the carboxyl terminus, and it has further been shown that these aberrant protein sequences are results of frameshift mutations which probably occur at the transcriptional level or by posttranscriptional editing of RNA (F.W. van Leeuwen et al, Science,vol 279, pp. 242-247).

In the case of βAPP two frameshift mutations have been observed, one by deletion of the di-nucleoside deoxyguanosine-deoxyadenosine (GA) unit from the (ACC)GAGAGAGA(ATG)$_A$ sequence (SEQ ID NO:11) in exon 9, and one by deletion of a GA unit from the (CAT)GAGAGA(ATG)$_A$ sequence (SEQ ID NO:12) in exon 10. The mutant βAPP peptides resulting from these frameshift mutations are shown in table 1. The peptides with seq id nos 1 and 4 are the mutant part of the βAPP protein sequence and the peptides with seq id nos 2, 3 and 5 represent mutant peptides extended into the normal βAPP sequence at the amino terminus.

normal βAPP;	RLEAKHRERMSQVMREWEEAERQAKNLPK (SEQ ID NO:13)
seq id no 1;	NVPGHERMGRGRTSSKELA
seq id no 2;	RLEAKHRENVPGHERMGRGRTSSKELA
seq id no 3;	RLEAKHRENVPGHERMG
seq id no 4;	MGRGRTSSKELA
seq id no 5;	ERMSQVMRMGRGRTS

Table 1.

Also in the case of Ubi-B two frameshift mutations have been observed, one by deletion of the di-nucleoside deoxyguanosine-deoxythymidine (GT) unit from the (TCT)GAGAGGT(GGT)$_\Lambda$ sequence (SEQ ID NO:14) in exon, and one by deletion of a di-nucleoside deoxycytosine-deoxythymidine (CT) unit from the (TCA)CTCT(GGA)$_\Lambda$ sequence (SEQ ID NO:15) in exon 3. The mutant Ubi-B peptides resulting from these frameshift mutations are shown in table 2. The peptides with seq id nos 6 and 9 are the mutant part of the Ubi-B protein sequence and the peptides with seq id nos 7, 8 and 10 represent mutant peptides extended into the normal Ubi-B sequence at the amino terminus.

normal Ubi-B;	HLVLRLRGGMQIFVKTLTGKTITLEVEPSD (SEQ ID NO:16)
seq id no 6;	YADLREDPDRQDHHPGSGAQ
seq id no 7;	HLVLRLRGYADLREDPDRQDHHPGSGAQ
seq id no 8;	HLVLRLRGYADLREDPD
seq id no 9;	GGGAQ
seq id no 10;	TLTGKTITGGGAQ

Table 2.

The mutant βAPP and Ubi-B proteins are only encoded for by cells in which corresponding frameshift mutations have occurred and are therefore targets for specific immunotherapy of Alzheimer's disease and Down syndrome.

According to the present invention, peptides corresponding to mutant βAPP and mutant Ubi-B proteins can be used to elicit T cellular immunity and specific killing of cells producing mutant βAPP and mutant Ubi-B proteins, which in Alzheimer's disease and Down syndrome patients are correlated with neuronal dysfunction leading to dementia.

of immunostimulatory DNA sequences (ISS). These can take the form of hexameric motifs containing methylated CpG, according to the formula : 5'-purine-purine-CG-pyrimidine-pyrimidine-3'. Our DNA vaccines may therefore incorporate these or other ISS, in the DNA encoding the peptides, in the DNA encoding the cytokine or other co-stimulatory molecules, or in both. A review of the advantages of DNA vaccination is provided by Tighe et al (1998, *Immunology Today*, 19(2), 89-97).

In one embodiment, the DNA sequence encoding the mutant βAPP and mutant Ubi-B peptides comprises:

Normal βAPP gene sequence (exons 9 and 10).

repeat 1

GAG AGG CTT GAG GCC AAG CAC C**GA GAG AGA** ATG TCC CAG GTC ATG (SEQ ID NO:17)

repeat 2

AGA GAA TGG GAA GAG GCA GAA CGT CAA GCA AAG AAC TTG CCT AAA (SEQ ID NO:18)

Mutant βAPP gene sequence, GA deleted from repeat 1.

GAG AGG CTT GAG GCC AAG CAC C**GA GAG A**AT GTC CCA GGT CAT GAG (SEQ ID NO:19)
AGA ATG GGA AGA GGC AGA ACG TCA AGC AAA GAA CTT GCC TAA

Mutant βAPP gene sequence, GA deleted from repeat 2.

GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG
AGA ATG GGA AGA GGC AGA ACG TCA AGC AAA GAA CTT GCC TAA (SEQ ID NO:20)

Normal Ubi-B gene (exon) sequence.

deletion motif

CAC CTG GTC CTG CGT CT**G AGA GGT** GGT ATG CAG ATC TTC GTG AAG
ACC CTG ACC GGC AAG ACC ATC ACC CTG GAA GTG GAG CCC AGT GAC (SEQ ID NO 21)

Mutant Ubi-B gene sequence, GT deleted from the deletion motif.

CAC CTG GTC CTG CGT CTG AGA GGG TAT GCA GAT CTT CGT GAA GAC
CCT GAC CGG CAA GAC CAT CAC CCT GGA AGT GGA GCC CAG TGA (SEQ ID NO:22)

Normal Ubi-B gene (exon 2) sequence.

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG (SEQ ID NO:23)

CT repeat

ACC CTG ACC GGC AAG ACC ATC ACT CTG GAG GTG GAG CCC AGT GAC (SEQ ID NO:24)

Mutant Ubi-B gene sequence, CT deleted from the CT repeat.

CAC CTG GTC CTG CGT CTG AGA GGT GGT ATG CAG ATC TTC GTG AAG
ACC CTG ACC GGC AAG ACC ATC ACT GGA GGT GGA GCC CAG TGA (SEQ ID NO:25)

The invention further encompasses vectors and plasmids comprising a DNA sequence encoding at least one frameshift mutant βAPP and/or Ubi-B peptide. The vectors include, but are not limited to *E.Coli* plasmid, a Listeria vector and recombinant viral vectors. Recombinant viral vectors include, but are not limited to orthopox virus, canary virus, capripox virus, suipox virus, vaccinia, baculovirus, human adenovirus, SV40, bovine papilloma virus and the like comprising the DNA sequence encoding a mutant βAPP and/or Ubi-B peptide.

It is considered that a treatment for Alzheimer's disease and Down syndrome, or prophylaxis for Alzheimer's disease, may be achieved also through the administration of an effective amount of a recombinant virus vector or plasmid comprising at least one insertion site containing a DNA sequence encoding a frameshift mutant peptide to a patient,